1167 Myocyte Function

Wednesday, April 1, 1998, 9:00 a.m.–11:00 a.m.
Georgia World Congress Center, West Exhibit Hall Level
Presentation Hour: 9:00 a.m.–10:00 a.m.

1167-1 Alterations in Cardiac Sarcoplasmic Reticulum Ca^{2+} Releasing Channels (ryanodine Receptors) of the Atrial Myocardium in Patients With Atrial Fibrillation

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Background: Atrial fibrillation (AF) is the most frequently encountered arrhythmia in the clinical setting. Recent studies have indicated that an inability to maintain intracellular Ca^{2+} homeostasis with a consequent increase in membrane-triggered activity could be the primary initiating factor of AF in some circumstances, and cytosolic Ca^{2+} abnormality is an important mediator of sustained AF. On the basis of these evidences, there is the possibility of alterations in SR Ca^{2+} regulatory proteins, which play an important role in the regulation of intracellular Ca^{2+}, in atrial myocardium with AF. The purpose of this study was to determine whether patients with AF have alterations in SR Ca^{2+} releasing channels (ryanodine receptors, RyRs) in atrial myocardium.

Methods and Results: To determine whether the number of cardiac RyRs changes in atrial myocardium, we measured the density (Bmax) and affinity (Kd) of [^{3}H] ryanodine binding sites in the right (RA) and/or left atrial (LA) myocardium removed during cardiac surgery from 12 patients with AF due to mitral valvular disease (MVD) and from 8 patients with thoracic aortic aneurysm and normal sinus rhythm (NSR). The Bmax of LA (0.17 ± 0.03 pmol/mg) was significantly lower than that of RA (0.21 ± 0.04, $p < 0.05$) in patient with MVD, and these levels were also significantly less than that of NSR (0.28 ± 0.08, $p < 0.05$). There was a significant negative correlation between the Bmax of LA and LA diameter ($r = -0.62$, $p < 0.05$), mean pulmonary artery pressure ($r = -0.65$, $p < 0.05$), or pulmonary capillary wedge pressure ($r = -0.68$, $p < 0.05$). There was no significant difference in Kd between the experimental groups.

Conclusion: These results suggest that in patients with MVD, mechanical overloads of atrial myocardium decreased the Bmax of RyRs, which might result in the impairment of intracellular Ca^{2+} handling and alter electrophysiologic properties of atrial myocardium as a cause of AF.

1167-2 Growth Hormone Enhances Cardiac Performance and Sarcoplasmic Reticulum Ca^{2+} Releasing Channels (ryanodine Receptors) in Cardiomyopathic Hamsters

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Background: Growth hormone (GH) has been shown to improve cardiac function in experimental model of heart failure and human dilated cardiomyopathy. The aim of this study was to clarify the effects of GH on cardiac function and cardiac sarcoplasmic reticulum (SR) Ca^{2+} releasing channels (ryanodine receptors, RyR) in the hearts of UM-X7.1 cardiomyopathic hamsters (U) during the development of heart failure.

Methods and Results: U (n = 9) and healthy control hamsters (C, n = 6) at the age of 20 wks were examined. Recombinant human GH (2 mg/kg/day SC) or vehicle was administrated for 3 wks in both hamsters. We examined the in vivo left ventricular (LV) size and contractile state with transthoracic echocardiography. To determine whether the number of cardiac RyRs changes in GH treated hamsters, we compared the density (Bmax) and affinity (Kd) of [^{3}H] ryanodine binding sites. The U treated with vehicle exhibited significant increases in the LV end-diastolic diameter (LVEDd, 6.1 ± 0.6 mm) and end-systolic diameter (LVESd, 5.2 ± 0.8), and significant decrease in LV fractional shortening (FS, 15 ± 5%) compared with C (4.6 ± 0.2, 2.5 ± 0.2 mm, 46 ± 3%, respectively). In the U, treatment with GH attenuated the increase of LVESd (4.6 ± 0.5, $p < 0.05$) and increased LVFS (23 ± 4, $p < 0.05$). Equilibrium binding assay of high affinity sites for [^{3}H] ryanodine showed that the U hamsters treated with vehicle exhibited marked decrease in Bmax (0.36 ± 0.04 pmol/mg, $p < 0.05$) compared with C (0.47 ± 0.03), whereas treatment with GH attenuated the reduction of Bmax in U (0.42 ± 0.03, $p < 0.05$) compared with vehicle-treated group. There was no significant difference in Kd between the experimental groups. In C, GH administration of this dose did not significantly enhance cardiac performance and density of RyRs. There was no significant difference in the connective tissue volume fraction by videodensitometry between the GH- and vehicle-treated groups in U (10.3 ± 0.2 vs 10.5 ± 0.3%).

Conclusions: These results suggest that GH might improve cardiac function by preserving the density of RyRs and maintaining SR function in cardiomyopathic hamster hearts.

1167-3 Results of Knockout Mouse Suggest Impaired Phosphorylation of Triplet CUG Binding Protein Pivotal to Pathogenesis of Cardiac and Skeletal Muscle Dysfunction

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The molecular defect responsible for dilated cardiomyopathy (DCM) remains elusive. Despite several loci having been mapped for DCM, no gene has been identified. In DCM of muscular dystrophies, while genes have been found, the responsible molecular mechanism is unknown. In myotonic dystrophy (DM) the genetic defect, excess triplet repeats (CTG) in the 3' end of myotonin protein kinase (Mt-PK), is known; how it induces the disease including DCM is yet to be determined. We identified a novel protein (CUG-BP) which binds specifically to the CUG repeats in the Mt-PK mRNA (Hum Mol Genet 1996; 5: 115). CUG-BP is phosphorylated by Mt-PK which transports the mRNA and other mRNAs with CUG repeats to the cytoplasm for their translation. We postulate the increased triplet repeats sequesters CUG-BP or inhibits phosphorylation by Mt-PK. Electrophoretic mobility shift analysis and immunoblotting with specific antibodies of whole cells, nuclear, and cytoplasmic extracts, normal cardiac and skeletal muscle exhibited hyperphosphorylated CUG-BP in the cytoplasm with only a trace of hypophosphorylated CUG-BP in the nucleus. In contrast, cardiac extracts from patients homozygous for DM, CUG-BP was hypophosphorylated and in the nucleus rather than the cytoplasm. The Mt-PK gene was eliminated in the mouse by homologous recombination resulting in a phenotype of cardiac and skeletal muscle weakness. Analysis showed cardiac CUG-BP, like in DM, was hypophosphorylated and almost exclusively in the nucleus rather than the cytoplasm. This strongly supports the hypothesis that multiple CUG triplet repeats, due to lack of phosphorylation, decreases expression of Mt-PK mRNA in the heart resulting in impaired contraction a dilated ventricle.

1167-4 Autocrine and Paracrine Effects of Stretch on Expression of Immediate-Early Genes and Atrial Natriuretic Peptide in Myocytes

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Background: Passive stretch of myocardial tissue, induced by left ventricular overload, initiates a growth response in this tissue. We investigated whether cardiac myocytes respond to stretch by autocrine and/or paracrine mechanisms.

Methods: Neonatal rat heart myocytes were grown on silicone elastomer plates and stretched by 15% for 0–60 min to study autocrine responses, or incubated for 30 min with conditioned medium (CM) derived from myocytes that were stretched for 0–60 min to study paracrine responses. At the times indicated RNA was isolated and hybridized in Northern blot analysis with probes of immediate-early (IE) genes, such as c-*fos*, c-*jun* and *fra*-1, and atrial natriuretic peptide (ANP).

Results: Autocrine responses. In stretched cells, c-*fos* expression increased rapidly (to 225% after 30 min) and transiently, c-*jun* expression had diminished to 86% and 71% after 45 and 60 min, resp. Also *fra*-1 expression decreased to 70% after 15 min. No change in ANP expression was found. *Paracrine responses.* In incubated with CM from 15–60 min stretched myocytes, expression of c-*fos*, c-*jun* and *fra*-1 had diminished. However, if incubated with CM from 30 and 45 min stretched myocytes, ANP expression increased to 257% and 308%, resp.

Conclusion: Stretched myocytes appear to secrete a factor that antagonizes c-*fos*, c-*jun* and *fra*-1 expression and stimulates ANP expression in stationary myocytes by paracrine mechanisms. The secreted factor has probably also a role in autocrine mechanisms.

1167-5 Alternative Pathway of Angiotensin II Production: Role of Human Chymase in Left Ventricular Remodeling

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Background: An alternative ACE-independent pathway of Angiotensin II (AT II) production, responsible for 80% of AT II synthesis in human hearts, is promoted by Human Chymase (HChy). This protease has been recently studied in patients with hypertension for the evaluation of its clinical effects. The aim of this study is to evaluate the possible role of HChy in myocardial tissutal alterations, in presence of various ventricular remodeling patterns.